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Effect of Early Maternal Deprivation with Chronic Stress on Behavior of Rats: It's Relation to Age and Sex

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ABSTRACT

The aim of this study was to investigate the influence of maternal deprivation with different stressors on behavior of male and female rats. Neonatal rats were isolated daily for 5 hr from postnatal day (PND) 1-27. Beginning on PND 27, the animals of maternal deprivation group were divided into four experimental groups as follows: subcutaneous injection of sodium chloride 0.9% and handling stress (I+H), subcutaneous injection of sodium chloride 0.9% and handling with noise exposure (I+N), noise exposure (N) and not stressed (NS) for ten days. The rats were tested for anxiety- related behavior using the elevated plus maze and open field on days 27 and 57. The (I+H) group showed higher locomotor activity and lower anxiety behavior as compared with other isolated groups on days 27 and 57. Reversely (I+N) group had the lowest locomotor activity and highest anxiety between experimental groups. Also male rats had lower total crossing than females in (I+H) and (I+N) groups on day 57($p<0.05$). These findings indicated that maternal deprivation may amplify anxiety-related behaviors and male rats more affected than females from some stressors (I+H and I+N) on the age of 57 days($p<0.05$). Also among different stressors were used in this study, administration of sound with injection more induced anxiety behavior in the isolated animals.

Key words: maternal deprivation- chronic stress- age- sex- rat.

INTRODUCTION

The early postnatal environment is seen as providing the potential for altering vulnerability to psychiatric disorders such as addiction, posttraumatic stress disorders (PTSD) and depression (1, 2). Exposure to stress early in life can induce an increased vulnerability to mood disorders later in life. Indeed, the origin of many adult diseases such as depression, anxiety, or impulse control disorders, can be found in infancy (3). The rat is an appropriate animal model to study early life psychiatric disorders (1, 2). There is growing evidence that stress during prenatal and postnatal periods of life can modify adaptive capacities in adulthoods.

It has been confirmed the environmental events can change and adapt the growth of rudimentary adaptive responses to stress. According to findings, differences in biological responses to stimuli threatening homeostasis in adult can be effected by even the fewest variations in the natal environment which change the process of development in brain systems(5).

It is established we have long-lasting changes of the hypothalamic-pituitary- adrenal (HPA) axis by adverse lifestyle in childhood which produce impressionable persons against stress that is placed in high risk group for mental disorders (6, 7).

Generally, the emotional behavior and normal response to stress is clearly shaped by the relationship between mother and infant and their lifestyle, therefore maternal deprivation (MD) is known as an important factor for infants which make them vulnerable for mental disorders. Since the maternal deprivation affects the processes of brain development and alters structural, neuroendocrine and emotional responses in adult, it seems the hypothalamic-pituitary-adrenal (HPA) axis has been obviously affected by maternal deprivation (4, 8).

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It has been shown that, in Wistar rats, a single prolonged episode of maternal deprivation (MD) during the neonatal period [24 h, postnatal day (PND) 9—10] induced, in the adulthood, diverse behavioral alterations that resemble specific symptoms of schizophrenia. Moreover, at adolescence, MD rats showed depressive-like behavior and altered motor responses. According to the neurodevelopmental hypothesis, certain behavioral abnormalities observed in MD animals may be related to altered neurodevelopmental processes. In fact, we have recently described sex-dependent alterations in developing hippocampal and cerebellar neurons and glial cells in MD neonatal rats, with males being more markedly affected (9). Stressful events in early life are involved in behavioural alterations in adulthood. Meaney and colleagues showed that neonatal handling of rat pups during the first 3 weeks of life was able to improve the spatial learning ability of aged animals in a water-maze task (10). Early postnatal handling induces a decrease in corticosterone secretion in response to stress (10, 11, 12) and an increase in the number of hippocampal receptors in adult rats (13). Postnatal handling protected from the age-related neuroendocrine and behavioural alterations. Handling in the early life, has been reported to prevent the change in behavioral reactivity observed in adult rats previously submitted to a prenatal stress (14). Postnatal handling has no effect on the performance of adult animals in a spatial memory task (the water maze) but improves performance during senescence. (10).

Considering the lack of data related to the effect of maternal deprivation with chronic stress in male and female rats, we performed this study to evaluate the MD and chronic stress effect on behavior of rats and their relation to age and sex.

MATERIALS AND METHODS

Experimental animals:

We use both young male and female Wistar rats in this study and all of them were kept on a 12 hours dark cycle (lights on from 07:00 to 19:00) at 22±2 C and we provide suitable amount of water and food for them.

Maternal separations

On the first day after birth (postnatal day-PND 1) we culled litters to 12 pups (6 males and 6 females) and they were divided randomly into two groups: maternal separation (PND1- PND27), and non-maternal separation. The separated litters were kept in their cage and their mothers transferred to other place and after five hours the mother was returned to home cage. The separation was done every day from 8:00 till 13:00. In control group, the litters were kept with their mothers till PND 27.

Variable chronic stress:

Beginning on PND 27, the animals of maternal deprivation group were divided into four experimental groups of 12 rats as follows: subcutaneous injection of sodium chloride 0.9% and handling stress (I+H), subcutaneous injection of sodium chloride 0.9% and handling with noise exposure (I+N), noise exposure (N) and not stressed(NS) for 10dats.

Handling stress

The animals of group 1 were handled daily for 15 min. when the rats were handling, they were picked up from the home cage and held in the hands for 15 min.

Sound stress:

Noise group were subjected to 100 dB SPL broadband white noise, 3 min daily for 4 weeks. The noise was produced by one loudspeakers (15W), driven by a white-noise generator (350 Hz), and installed 30 cm above the cage.

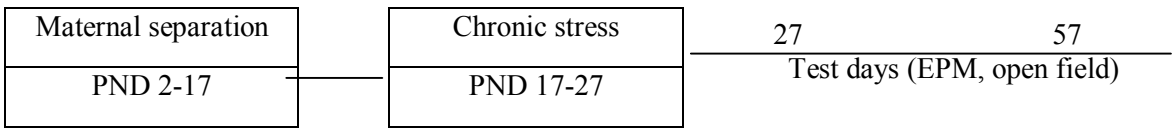
Elevated plus maze test

Elevated plus maze test were don for animals on days: 27, 37, 57 and 67. the apparatus consisted of two open arms(5×30 cm) and two closed arms (5×30×15 cm) radiating from a central platform(5×5cm) to form a plus sign figure. The apparatus was situated 40 cm from the floor. The open arm edges were 15 cm in height. The animals were individually examined in 5-min sessions in this apparatus. Each rat was placed in the central platform facing one open arm. The percentage of time spent in the open arms was calculated for each rat.

Open field

The open field test was done on days: 27, 37, 47, and 57. The open field consists of a wooden box 90.00 cm× 90.00 cm × 38.00 cm, positioned in a dimly lighted room. The walls are painted black lines into 25 squares 17.0 cm× 17.0 cm. rats are placed at the corner of the open field for the following 3 min, the number of line crossing and the time spent in the central and the peripheral areas are manually recorded. The total crossings represent the activity level of the rat. The ratio between line

crossings in the peripheral area and the total line crossings, and the relative time spent, are considered a measure of anxiety (4).



1: handling-injection stress
2: noise stress
3: sound and injection stress

Experimental design

Statistical analysis:

Two- way ANOVA was used for statistical comparisons. Each ANOVA reporting significant effects was followed by Tueky's post hoc test. Significant was set at $p<0.05$.

RESULTS

Open field test:

Fig.1 depicts total crossing in the 6 groups of rats on day 27. Means of total crossing in isolated groups, F: 47.44±4.52, M: 46.5±4.21 (I+H); F: 33.42±3.76, M: 33.5± 3.75(I+N); F: 36.5±2.74, M: 37 (N); F: 44.22±3.12, M: 43.5±4.11 (NS), significantly decreased as compared to control group (F: 71.5±3.76, M: 70.21±4.78), ($P<0.01$).

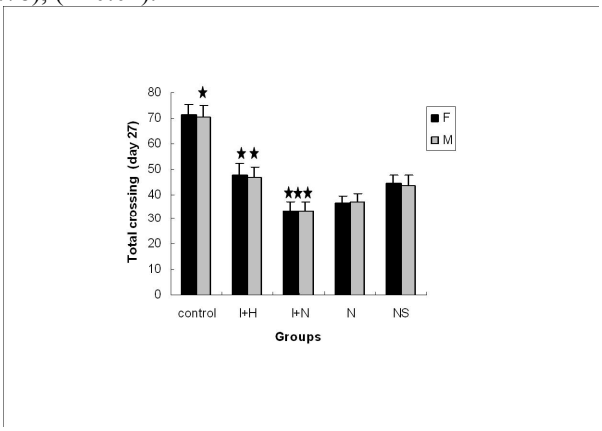


Fig.1: total crossing of experimental groups on days 27. (I+H) handling and injection stress, (I+N) injection with noise stress, (N) noise stress, (NS) not stressed. * $p<0.01$ (I+H), (I+N), (N) and (NS) as compared with control group . ** $p<0.05$, between (I+H) as compared with (I+N), (N) and (NS) groups.*** $p<0.05$, between (I+N) as compared with (I+H), (N) and (NS) groups.

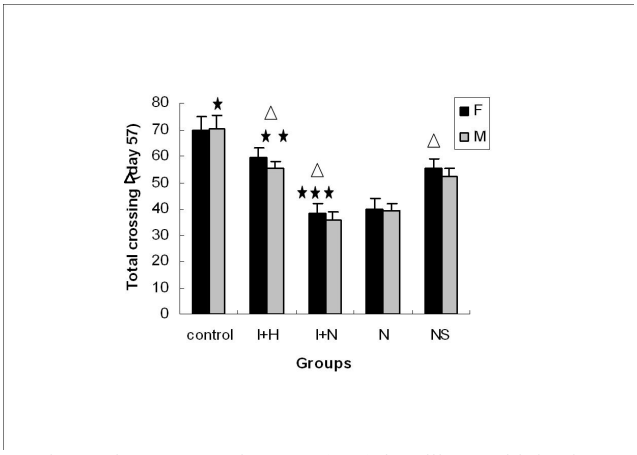


Fig.2: total crossing of experimental groups on days 57. (I+H) handling and injection stress, (I+N) injection with noise stress, (N) noise stress, (NS) not stressed. * $p<0.01$ (I+H), (I+N), (N) and (NS) as compared with control

group. ** p<0.05, between (I+H) as compared with (I+N), (N) and (NS) groups.*** p<0.05, between (I+N) as compared with (I+H), (N) and (NS) groups. P<0.05 between male and female groups.

Fig.2 shows mean ±SD of total crossing in various groups of rats on days 57. Similarly Mean total crossing significantly decreased in isolated groups: F: 59.4±3.46, M: 56.9±2.84 (I+H); F: 38.34±3.55, M: 36.22± 3.25(I+N); F: 40.12±4.15, M: 39.5±2.78 (N); F: 55.26±3.86, M: 53.39±3.2 (NS), significantly decreased when comparing to control group (F: 69.64±5.36, M: 70.2±4.93) (P<0.01).

Elevated plus maze test (EPM):

The mean ± SD of the time spent in the open arms between isolated groups on day 27 was (F: 24.13±3.52, M: 24.56±3.67) in I+H, (F: 17.42±3.21, M: 15.45±3.12) in I+N, (F: 19.34±3.40, M: 18.87±2.75) in N and (F: 22.67±4.35, M: 19.33±3.21) in NS. Significant increase was seen in (I+H) group as compared with other isolated groups. The time spent in the open arms significantly decreased in I+N group (p<0.01) (Fig.3).

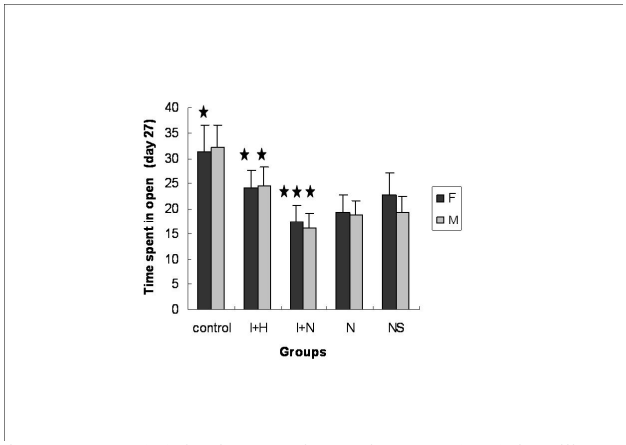


Fig.3: the time spent in the open arm (%) in the experimental groups.(I+H) handling and injection stress, (I+N) injection with noise stress, (N) noise stress, (NS) not stressed. * p<0.01 (I+H), (I+N), (N) and (NS) as compared with control group . ** p<0.05, between (I+H) as compared with (I+N), (N) and (NS) groups.*** p<0.05, between (I+N) as compared with (I+H), (N) and (NS) groups.

Similar pattern was seen on day 67 in anxiety behavior of the animals at the EPM test. The animals of the (I+H) group spent more time in the open arms (F: 27.46±3.67, M: 26.66±3.12) when compared to the other isolated groups: (p<0.01) being (F: 18.54±2.77, M: 19.1±3.44) in I+N, (F: 22.39±2.67, M: 21.9±2.27) in N and (F: 24.86±2.77, M: 26.74±3.15) in NS. Reversely the time spent significantly decrease in I+N as compared to the other isolated groups (p<0.05) (Fig.4).

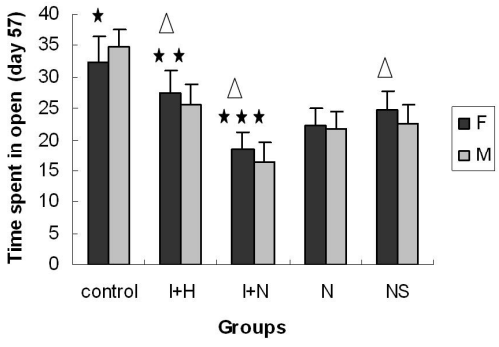


Fig.4: the time spent in the open arm (%) in the experimental groups. (I+H) handling and injection stress, (I+N) injection with noise stress, (N) noise stress,(NS) not stressed. * p<0.01 (I+H), (I+N), (N) and (NS) as compared with control group . ** p<0.05, between (I+H) as compared with (I+N), (N) and (NS) groups. *** p<0.05, between (I+N) as compared with (I+H), (N) and (NS) groups. P<0.05 between male and female groups.

DISCUSSION

In this study, we examined the effect of maternal deprivation and chronic stress on the behavior of rats and its relation to age and sex. The present study showed that maternal deprivation and chronic stress alter the behavior of the animals and the changes depend on the age and the sex of animals. Among different stressors were used in this study, administration of sound with injection more decreased locomotor activity and more increased anxiety in the isolated animals. Additionally male rats were more sensitive than female in the adult age.

Mother infant interactions during early postnatal life are crucial for the proper maturation of the central nervous system and appear to be relevant for the development of emotionally and for the vulnerability to psychiatric disorders (6). Traumatic events in early life are associated with an increased risk of psychiatric disease in adulthood (15). Avi et al. showed that an exposure of rats to a relatively brief stressful experience during their 4^{th} week of life has profound and long-lasting behavior effects (16). Knuth et al. study showed that neonatal isolation may enhance anxiety- related behavior, especially in response to stress. (17). There is increasing awareness that events in early life may have persistent influence on the physiology and behavior in the adults. Prolonged (3 h) maternal separation of pups from the dam on PND 2-14 impaired performance in the Morris water maze task relative to their respective control groups. Kosten et al. findings demonstrated that the early life manipulation of neonatal isolation leads to enduring effects on hippocampal function resulting in impaired memory performance and modifications in hippocampus (19) According to findings it is confirmed the ability of GRs in different neural structures such as hippocampus is disturbed by early life stress. There is an obvious decrease of fore brain GRs and abnormal HPA axis suppressing effects of the synthetic glucocorticoid , dexametasone in adult rats that experience maternal separation in their childhood(4).

In this present study we found that isolated groups had lower locomotor activity and higher anxiety behavior than control groups on the age of 27 and 57. These findings are similar to those of neonatal isolation effect studies (17, 19 and 4). Also the (I+H) group showed higher locomotor activity and lower anxiety behavior as compared with I+N, N and NS. Reversely I+N group had lowest locomotor activity and highest anxiety between isolated groups. This finding indicated that neonatal handling attenuated harmful effects of maternal deprivation but combination of handling with noise stress increased anxiety behavior in the isolated groups. Our results showed that there is significant difference between male and female behavior in adult age but mechanism of which isn't clear and more studies should be done to prove this problem.

5-hydroxy tryptamine (5-HT, serotonin) is an important modulator neurotransmitter which has a major role in the control of emotional and stress responses (18). In this study we didn't evaluate changing in 5-HT levels. We suggest that moderate stress (postnatal handling) attenuated anxiety induced by maternal deprivation and adult male rats were sensitive than female to early life stressors.

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